CHEMOIMMUNOTHERAPY OF METASTATIC LARGE BOWEL CANCER

Nonspecific Stimulation with BCG and Levamisole

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The administration of two chemoimmunotherapy programs to 103 consecutive patients with metastatic colorectal cancer resulted in improved survival for patients who achieved either objective tumor regressions or disease stabilization for more than 8 weeks. Objective tumor regression was observed in 47% of patients treated with the Ftorafur-methyl-CCNU-methotrexate-Bacillus Calmette-Guerin (FTOR-MeM-BCG) program and in 34% of patients treated with the 5-fluorouracil-methotrexate-Baker's antifol (FU-M-BAF) ± Levamisole program. The combined median duration of survival for patients who achieved objective tumor regression and disease stabilization with FTOR-MeM-BCG was 13 months compared with 6 months for patients who had progression of disease (p = 0.001). The corresponding values for patients treated with FU-M-BAF ± levamisole were 11 months and seven months, respectively (p = 0.001). While the role of BCG immunotherapy in these results remains speculative, the administration of levamisole immunotherapy did not appear to have influenced results significantly. Patients who presented at diagnosis with Dukes A, B and C lesions, and therefore had longer disease-free intervals, responded more frequently to chemoimmunotherapy and survived longer than patients who presented at diagnosis with Dukes D lesions. Similarly, greater antitumor effect was observed in patients with lower pretreatment plasma CEA levels. Evaluation of these pretreatment characteristics may have significant implications for the design of future clinical trials.

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THE RESULTS OF AVAILABLE TREATMENTS FOR patients with metastatic colorectal cancer are poor. A few chemotherapeutic agents, mainly 5-fluorouracil (5-FU) and the nitrosoureas administered alone or in combination, have demonstrated some degree of antitumor activity, although with minimal or no bearing on patients' survival.[1,4,5,16,19] Thus, there is a need to continue the active investigation of newer antitumor agents and newer treatment modalities for these patients.

Accordingly, we have recently studied two chemoimmunotherapy programs in patients with advanced colorectal cancer. The programs included the administration of two newer antitumor agents, Ftorafur (FTOR) and Baker's antifol (BAF), and that of nonspecific immunotherapy with Bacillus Calmette-Guerin (BCG) and Levamisole, in view of the encouraging preliminary clinical results with these compounds.[7-10,12,17-18,21-22,24-25] The results of our experience with these programs are presented.

PATIENTS AND METHODS

This study was conducted in a consecutive series of 103 adult patients with proven metastatic cancer of the large bowel and rectum who were referred to the Department of Developmental Therapeutics of The University of Texas System Cancer Center M. D. Anderson Hospi-

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tal and Tumor Institute between January, 1974 and December, 1976. Most patients had disease that could be measured and were therefore evaluable for response to treatment. Patients whose disease was not clearly measurable, such as those with diffuse peritoneal involvement, ascites, or pelvic masses, were also included but were evaluable only for survival studies.

Patients were treated with either of two chemoimmunotherapy programs, which were evaluated in chronological sequence. The first program, FTOR-MeM-BCG, was conducted during the period from January 1974 and June 1975. It consisted of the administration of Ftorafur, methyl CCNU (Me) and methotrexate (M) combination chemotherapy and BCG immunotherapy. Ftorafur was given intravenously (i.v.) at a dose of 2 g/M^2/day for 5 days (days 2–6), each dose dissolved in 200 ml of 5% dextrose solution and administered over a 1-hour period. Methyl CCNU was given orally at a dose of 100 mg/M^2 on day 1, and methotrexate was given in a rapid i.v. infusion at a dose of 30 mg/M^2 dissolved in 50 ml of 5% dextrose on days 1, 8 and 15 of each treatment. Scarifications with the lyophilized Pasteur strain of BCG at a dose of 6 $\times$ 10^8 viable organisms were performed on days 7, 14 and 21 of each treatment. Courses of treatment were repeated every 4 weeks.

The second chemoimmunotherapy program, FU–M–BAF ± Levamisole, is part of an ongoing randomized clinical trial initiated in July 1975 in which patients received, in alternation, single agent chemotherapy with 5-FU and an antifolate (either methotrexate or BAF) with or without Levamisole immunotherapy. All patients initially received 5-FU i.v. at a dose of 500 mg/M^2/day for 5 days (days 1–5), repeated every 6 to 8 weeks depending upon recovery from toxicities. Each dose of 5-FU was dissolved in 200 ml of 5% dextrose solution and given over a 1-hour period. Patients randomized to receive methotrexate were given a 3-day dose of 15 mg/M^2 in a rapid i.v. infusion of 50 ml of 5% dextrose beginning 3 to 4 weeks after a course of 5-FU. Patients randomized to receive BAF were given a dose of 250 mg/M^2/day i.v. for 3 days instead of methotrexate. Each dose of BAF was dissolved in 200 ml of 5% dextrose solution and given over a 1-hour period. Three weeks after the administration of either antifolate, the cycle beginning with 5-FU chemotherapy was repeated. Levamisole immunotherapy was administered orally at a dose of 150 mg/M^2/day on days 7, 8, 14 and 15 of treatment with each chemotherapeutic agent.

Because of pharmacologic considerations, the initial doses of methotrexate and BAF were reduced for patients with impaired renal and hepatic functions, respectively.[2,11] Dose reductions were of the magnitude of 25% to 50% according to the patient's particular clinical situation. During subsequent treatments, the doses of all agents were adjusted to maintain a tolerable degree of hematologic (absolute neutrophil counts = $\simeq$750–1000/mm^3, platelet counts = $\simeq$75,000–100,000/mm^3) and nonhematologic toxicities. A signed consent form was obtained from each patient according to institutional policies.

Before and during treatment, patients underwent a careful evaluation to determine both the areas of tumor involvement and tumor response to treatment. Included were a complete physical examination and, in most patients, serial evaluations of blood counts, SMA-100, plasma levels of carcinoembryonic antigen (CEA) by the method of Egan *et al.*,[3] urinalysis, chest x-rays, liver–spleen scans, barium enemas and abdominal ultrasound examinations. The antitumor response to the treatment was assessed according to the following criteria: complete response, a complete disappearance of all evidence of disease; partial response, significant tumor shrinkage $\geq$50% (partial remission $\geq$50%) or between 25% and 50% (partial remission <50%) of the sum of the products of all measurable lesions without the appearance of new lesions. The category of disease stabilization included patients whose tumors achieved response less than partial remissions, and patients whose tumors did not change for a minimum of 8 weeks. Also included in this category were patients who achieved objective improvement in areas that were difficult to measure adequately, such as ascites, diffuse intra-abdominal carcinomatosis, and pelvic masses. Appearance of new lesions and enlargement of existing lesions indicated disease progression. Survival calculations for patients entering this study were determined from the point of initiation of treatment by the method of Kaplan and Meier for censored and uncensored data.[13] The differences between survival curves were evaluated by the one-tailed generalized Wilcoxon test according to Gehan.[6] Patients dying within 2 weeks after the onset of therapy were considered early deaths and were not evaluable for survival. All side effects associated with the administration of treatments were recorded and analyzed.

RESULTS

One hundred and three consecutive adult patients with metastatic cancer of the large bowel

TABLE 1. Characteristics of Patients with Advanced Colorectal Cancer Treated with Two Chemoimmunotherapy Programs

	FTOR-MeM-BCG	FU-M-BAF ± Lev.
No. patients entered	61*	42
No. early death patients	4	2
No. patients evaluable	57	40
Sex		
females	22	17
males	35	23
Age		
median	54 yr	55 yr
range	20–76 yr	15–71 yr

* Included were 23 patients who failed prior 5-FU therapy.
FTOR = ftorafur; Me = methyl CCNU; M = methotrexate; BCG = Bacillus Calmette-Guerin; FU = 5-fluorouracil; BAF = Baker's antifol; Lev. = levamisole.

and rectum were treated with two chronologically sequential chemoimmunotherapy programs over the 3-year period of the study (Table 1). Included were 61 patients treated with the FTOR-MeM-BCG program and 42 patients treated with the FU-M-BAF ± Levamisole program. With the exception of 23 patients (38%) who had failed to respond to 5-FU treatment prior to receiving FTOR-MeM-BCG, the characteristics of patients entering both programs were similar.

The antitumor response to both chemoimmunotherapy programs could be evaluated in 38 patients treated with FTOR-MeM-BCG and in 32 patients treated with FU-M-BAF ± Levamisole (Tables 2 and 3). In the remaining patients, because of difficulty in adequately measuring the areas of tumor involvement, the evaluation could only be made in terms of stabilization of disease or tumor progression. Eighteen of the 38 (47%) evaluable patients treated with FTOR-MeM-BCG responded. Response was slightly better for patients who did not receive prior chemotherapy (52%) than for patients who did (40%). Disease stabilization occurred in 10 (26%) patients with measurable lesions and in 13 of 19 (68%) patients whose lesions could not be adequately measured. Among 32 evaluable patients treated with FU-M-BAF ± Levamisole, the overall tumor response was 34%. Response was slightly better for patients treated with methotrexate (47%) than BAF (27%) ($p = 0.84$). Disease stabilization was achieved in 11 (34%) patients with measurable lesions and in three of seven (43%) patients whose lesions could not be measured adequately. The median point of maximal tumor response was 1.5 months for both chemoimmunotherapy programs. The median duration of tumor regressions for patients treated with FTOR-MeM-BCG was 6 months (range, 2 to 14 months) and for patients achieving disease stabilization, 5 months (range, 2 to 9+ months). To date, the median duration of response for patients treated with FU-M-BAF ± Levamisole is 3.5+ months (range, 1 to 7.5+ months). Independent of the antifolate administered, the addition of Levamisole immunotherapy did not appear to have influenced the response rate to FU-M-BAF chemotherapy (Table 4).

Fifty-three of the 70 patients with measurable disease had determinations of plasma CEA levels immediately prior to the onset of chemoimmunotherapy. The response of these pa-

TABLE 3. Response in Patients with Measurable Lesions in Relation to the Antifolate Administered

Response	FU-M ± Lev. No. pts.	%	FU-BAF ± Lev. No. pts.	%	Overall No. pts.	%
Complete	1	6	0	0	1	3
Partial > 50%	2	12	1	7	3	9
Partial < 50%	4	24	3	20	7	22
Overall	7	47	4	27	11	34
Stabilization	7	47	4	27	11	34
Progression	3	18	7	47	10	31
TOTAL	17		15		32	

TABLE 2. Response to FTOR-MeM-BCG of Patients with Colorectal Cancer and Measurable Lesions

Response	No prior RX No.	%	Prior RX No.	%	Overall No.	%
Complete	2	9	2	13	4	11
Partial > 50%	4	17	0	0	4	11
Partial < 50%	6	26	4	27	10	26
Overall	12	52	6	40	18	47
Stabilization	6	26	4	27	10	26
Progression	5	23	5	33	10	26
TOTAL	23		15		38	

TABLE 4. Response in Patients with Measurable Lesions in Relation to the Administration of Levamisole

Response	Levamisole No. pts.	%	No levamisole No. pts.	%
Complete	0	0	1	8
Partial > 50%	3	16	0	0
Partial < 50%	4	21	3	23
Overall	7	37	4	31
Stabilization	6	32	5	38
Progression	6	32	4	31
TOTAL	19		13	

TABLE 5. Response to Chemoimmunotherapy* Related to the Pretherapy Plasma CEA Values

CEA (ng/ml)	No. pts.	CR + PR	< PR	Overall (%)
< 10	21	5	6	11 (52)
11–100	10	2	2	4 (40)
> 101	22	1	3	4 (18)

* Includes data from the FTOR-MeM-BCG and the FU-M-BAF ± Levamisole studies.

TABLE 6. Response to Chemoimmunotherapy* Related to the Extent of Disease at Original Diagnosis

Staging (Duke's)	No. pts.	CR + PR	< PR	Overall (%)
A	1	0	0	0
B	17	4	5	9 (53)
C	23	3	7	10 (43)
D	25	4	2	6 (24)

* Includes data from the FTOR-MeM-BCG and the FU-M-BAF ± Levamisole studies.

tients to both chemoimmunotherapy programs was inversely related to the pretreatment levels of CEA (Table 5). Tumor regressions were seen in 52% of 21 patients whose initial CEA levels were less than 10 ng/ml and in 18% of 22 patients whose initial CEA levels were greater than 100 ng/ml ($p = 0.04$). Also, response to chemoimmunotherapy correlated inversely with the extent of the disease at original diagnosis (Table 6). Among 66 patients with measurable disease, responses were more common for those patients who had tumors of the Dukes' A, B or C class (48% of 40 patients) than among patients who were found to have Dukes' D class lesions (24% of 25 patients). However, this difference was not statistically significant ($p = 0.10$).

The survival rates for patients entering this

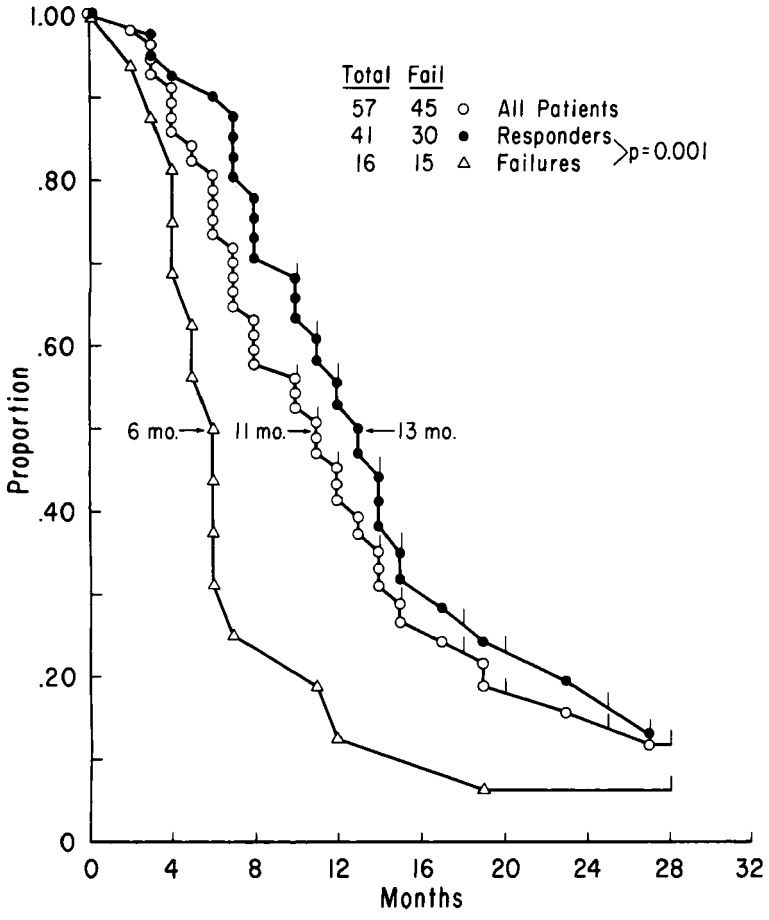

FIG. 1. Chemoimmunotherapy of advanced colorectal cancer with FTOR-MeM-BCG. Survival from onset of treatment.

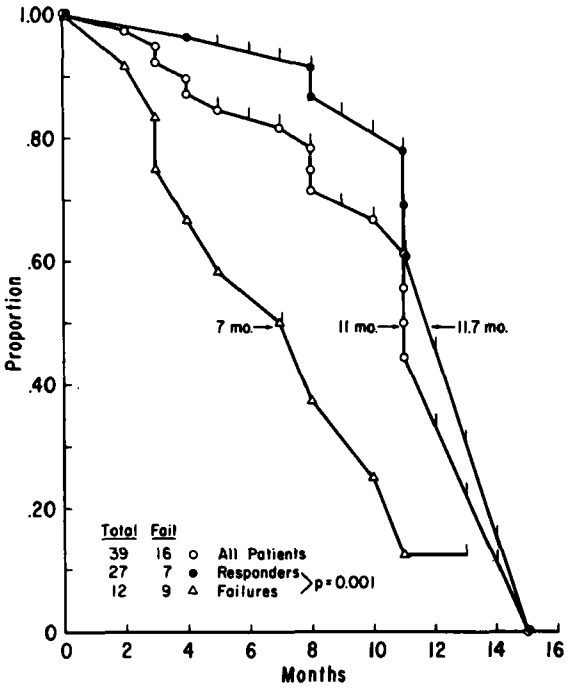

FIG. 2. Chemoimmunotherapy of advanced colorectal cancer with FU-M-BAF ± Levamisole. Survival from onset of treatment.

study are shown in Figs. 1–4. Seven patients who died early because of tumor progression, four in the FTOR–MeM–BCG program and 3 in the FU–M–BAF program, were excluded from these calculations. Since the survival rates for patients who achieved tumor regressions and for those who achieved disease stabilization were identical, the survival data for these patients were combined, and the patients were represented as "responders" in the figures; data for patients who had progression of disease were listed under "failures." The median duration of survival from onset of treatment for patients treated with the FTOR–MeM–BCG program was 11 months (Fig. 1). The median duration of survival for responding patients was longer than for patients who failed to respond to treatment (13 months vs 6 months; $p = 0.001$). Similarly, the median duration of survival for patients treated with the FU–M–BAF ± Levamisole program was 11 months (Fig. 2). Survival was also superior for patients who responded to therapy (11.7 months) than for patients who did not (7 months) ($p = 0.001$). The median duration of survival of the Levamisole-treated patients (Fig. 3) was not significantly longer than that for patients treated with chemotherapy alone (11 months and 9.9 months, respectively; $p = 0.16$). The survival for patients measured from onset of either chemoimmunotherapy program was also related to the extent of disease at original diagnosis (Fig. 4). Among 93 patients in whom this information was available, the median duration of survival was longer (12.3 months) for patients who, at diagnosis, were found to have Dukes' A, B or C type lesions than for patients who were found to have Dukes' D lesions (10 months) ($p = 0.04$). This difference was also reflected in the longer median duration of survival of patients who responded (14 months vs 11 months) and that of patients who failed to respond to treatment (7 months vs 5 months) (Fig. 5).

The toxicities associated with both chemoimmunotherapy programs were primarily hematologic and gastrointestinal (Table 7). The degree of leukopenia and thrombocytopenia was moderate, although more common in patients treated with FTOR–MeM–BCG. Thrombocytopenia was particularly more common, cumulative and pronounced as a result of this treatment. Nausea and vomiting were the most frequent gastrointestinal side effects and were also more common in patients who had received FTOR–MeM–BCG. Of particular importance was the development among these patients of

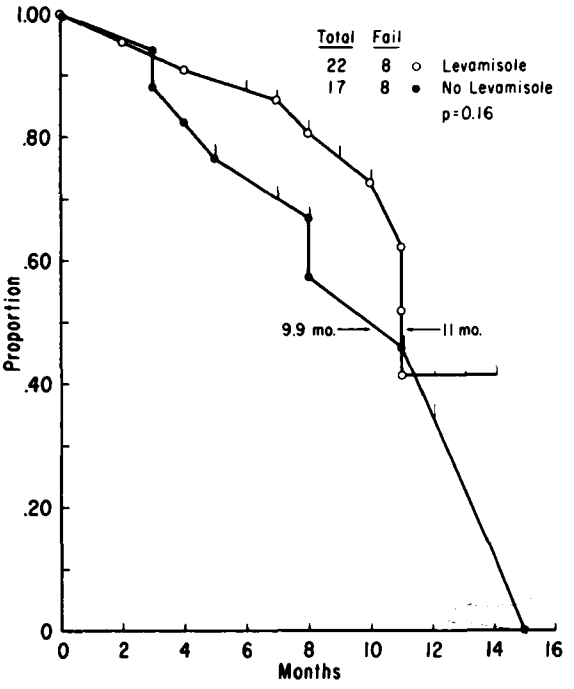

FIG. 3. Chemoimmunotherapy of advanced colorectal cancer. Effect of Levamisole on survival.

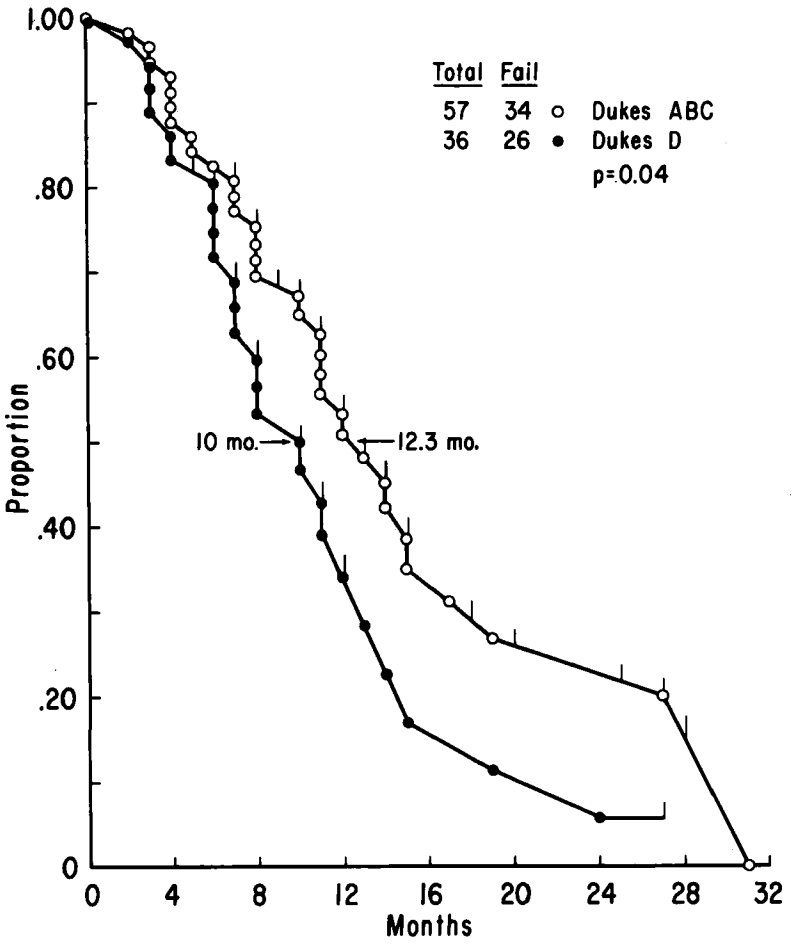

FIG. 4. Chemoimmunotherapy of advanced colorectal cancer. Survival from onset of treatment related to original staging according to Dukes.

transient episodes of weakness, dizziness and ataxia, which we have previously described for patients who received Ftorafur.[24]

DISCUSSION

The administration of two chemoimmunotherapy programs to patients with advanced colorectal cancer resulted in a moderate degree of improvement in the prognosis for these patients. These programs, the FTOR-MeM-BCG and the FU-M-BAF ± Levamisole, included several old and newer active chemotherapeutic agents administered in combination with BCG and Levamisole immunotherapy, respectively. In the FTOR-MeM-BCG program, full doses of two myelosuppressive agents (methyl-CCNU and methotrexate) were given in combination and a newer nonmyelosuppressive fluoropyrimidine, Ftorafur.[25] This compound is slowly metabolized into 5-FU *in vivo*, producing elevated plasma levels of 5-FU for a prolonged period of time, thus mimicking the less myelosuppressive continuous intravenous infusion schedule of 5-FU.[14,15,23] Nonspecific immune stimulation with BCG scarifications was added to chemotherapy because of the encouraging results previously reported from this institution in the treatment of patients with solid tumors and acute myelogenous leukemia (AML). BCG immunotherapy produced a significant prolongation of the disease-free interval and survival period for patients with Dukes' C class colorectal cancer who were receiving adjuvant therapy.[17,18] Prolongation of the duration of response and survival for patients with malignant melanoma,[9] breast carcinoma,[7] and AML[8] has also been reported. In

the FU-M-BAF ± Levamisole program, the chemotherapy strategy included the evaluation of sequential single agent chemotherapy at full doses and the comparison of methotrexate with a new antifolate, Baker's antifol. This compound has the advantage of not requiring an active transport system for intracellular incorporation and has shown significant antitumor activity in patients with prior exposure to methotrexate.[21] Immune restoration with Levamisole was also administered in view of the encouraging data derived from studies conducted in patients with lung cancer,[24] breast cancer,[12,22] and malignant melanoma.[10]

The overall tumor regression obtained with both programs was moderate, although greater for patients receiving FTOR-MeM-BCG (47%) than for patients receiving FU-M-BAF ± Levamisole (34%). However, tumor regression ≥50% was achieved in only 22% of patients treated with FTOR-MeM-BCG. These results do not compare favorably with others in the literature; responses ranging from 18% to 45% have been attributed to a variety of drug combinations, primarily 5-FU and methyl CCNU.[1,4,5,16,19] However, comparing these results, it appeared that patient's selection and criteria for the evaluation of tumor response may account for these differences. In fact, the survival rates for patients treated with these other regimens have not been improved and median survival times ranging from 6 to 8 months have remained unchanged.[1,4,5,16] By contrast, the survival rates for patients treated with our programs were influenced favorably. The median survival period of 11 months for patients treated

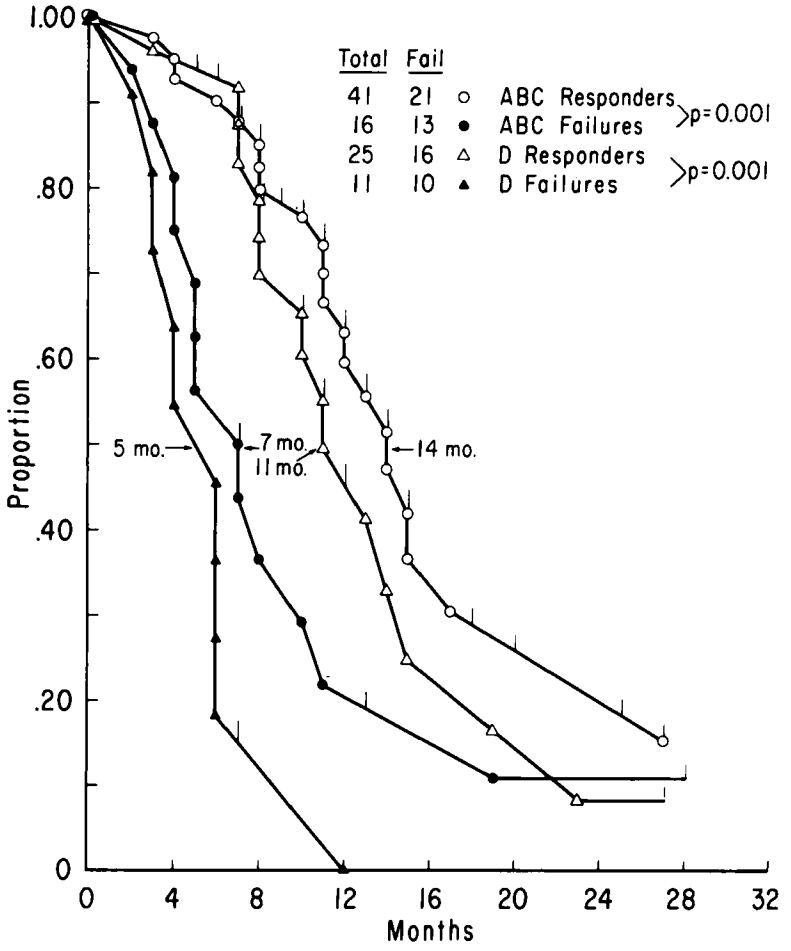

FIG. 5. Chemoimmunotherapy of advanced colorectal cancer. Survival from onset of treatment related to response and original staging according to Dukes.

TABLE 7. Toxicities Association with Both Chemoimmunotherapy Programs

Toxicities	FTOR-MeM-BCG	FU-M-BAF ± Lev. 5-FU	M	BAF
1. Hematologic				
Lowest leukocyte counts/mm³				
Median	3.3	3.7	4.2	5.2
Day	17	18	12	8
% < 1000	16	6	4	0
Lowest platelet counts mm³				
Median	91	220	256	244
Day	16	15	10	10
% < 50,000	20	1	0	0
2. Other*				
% Nausea & vomiting	70	14	2	2
% Stomatitis	15	17	5	5
% Diarrhea	3	8	1	1
% Infection	3	12	0	0
% Bleeding	2	0	0	0
% Weakness, dizziness, ataxia	15	3	0	1

* In addition, there were two episodes of myalgias and fever associated with BCG treatment, and two of fever associated with Levamisole therapy.

with both programs compares favorably with other results and reflects the improved survival for patients who achieved tumor regressions and disease stabilization. The combined median survival period for these two groups of patients was significantly better than that of patients who had disease progression in the FTOR-MeM-BCG program (13 months vs 6 months) and in the FU-M-BAF ± Levamisole program (11.7 months vs 7 months; p = 0.001).

The effect of nonspecific immunotherapy on the results of this study could only be ascertained for the patients treated with FU-M-BAF ± Levamisole, because some of these patients received chemotherapy alone. The response rate and the duration of response and survival were similar for patients who received Levamisole and for those who did not receive Levamisole. Thus, in this rather small number of patients, the administration of Levamisole immunotherapy did not appear to influence the results obtained with chemotherapy. In the group of patients treated with FTOR-MeM-BCG, we could not ascertain whether the improved survival was the result of administering BCG immunotherapy, combination chemotherapy or both. The encouraging results obtained with BCG in the adjuvant therapy of patients with colorectal cancer[17,18] may suggest a beneficial effect of this immunotherapeutic agent in our patients. Nevertheless, this is an area that requires further investigation.

The results of the present study suggest that the natural history of the disease influences the results of chemoimmunotherapy. Patients who had longer disease-free intervals from the time of original diagnosis (patients with Dukes' A, B or C class lesions) to the time of development of distant metastases (Dukes' D class) had better tumor responses and longer survival periods from initiation of chemoimmunotherapy. Tumor responses were obtained in 48% of 40 patients who originally presented with Dukes' A, B or C class lesions compared with 24% of 25 patients who originally presented with Dukes' D class lesions (p = 0.10). The impact of this difference becomes more apparent when analyzing the duration of survival for these patients. The median survival from the time chemoimmunotherapy started for patients who presented with Dukes' A, B or C class lesions was 12.3 months as opposed to 10 months for patients who presented with Dukes' D class lesions (p = 0.04). The response to chemoimmunotherapy was also related to the pretreatment plasma CEA levels. Fifty-two percent of 21 patients whose pretreatment CEA levels were <10 ng/ml responded to treatment compared with 18% of 22 patients whose pretreatment CEA levels were >100 ng/ml (p = 0.04). Similar observations on the importance of pretreatment plasma CEA levels have been made for patients undergoing initial surgical treatment.[20] These findings indicate that patients' pretreatment characteristics should be studied when analyzing results of chemotherapy in patients with metastatic colorectal cancer. The understanding of these variables may be helpful in designing future clinical trials, since they may assist in identifying those patients who are likely to respond to conventional treatment and patients who may benefit most from the administration of investigational treatments.

The administration of these chemoimmunotherapy programs to patients with metastatic colorectal cancer resulted in a modest improvement in the survival of most patients who achieved either objective tumor regressions or disease stabilization. The administration of Levamisole immunotherapy did not appear to have influenced these results significantly. The role of BCG immunotherapy remains speculative. Results of treatment were influenced by the disease-free interval prior to the onset of chemoimmunotherapy and the pretreatment plasma CEA levels. Attention to these pretreatment characteristics may be helpful in designing future clinical trials.

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